EASTERN REGIONAL RESEARCH CENTER
AGRICULTURAL RESEARCH SERVICE
UNITED STATES DEPARTMENT OF AGRICULTURE
600 E. MERMAID LANE
WYNDMOOR, PA 19038
(215) 233-6400

Title: Resuscitation of Acid-Injured *Salmonella* in Enrichment Broth, in Apple Juice and on the Surfaces of Fresh-Cut Cucumber and Apple

Author(s): C.H. Liao, and W.F. Fett,

Citation: Letters in Applied Microbiology (2005) 41: 487-492

Number: 7599

Resuscitation of acid-injured *Salmonella* in enrichment broth, in apple juice and on the surfaces of fresh-cut cucumber and apple*

C.-H. Liao and W.F. Fett

Eastern Regional Research Center, Agricultural Research Service, US Department of Agriculture, Wyndmoor, PA, USA

2005/0085: received 27 January 2005, revised 26 April 2005 and accepted 3 June 2005

ABSTRACT

C.-H. LIAO AND W.F. FETT. 2005.

Aims: To investigate the resuscitation of acid-injured *Salmonella enterica* in selected enrichment broths, in apple juice and on cut surfaces of apple and cucumber slices.

Methods and Results: Following exposure to 2·4% acetic acid for 7 min, *S. enterica* (serovars Mbandaka, Chester and Newport) cells were used to inoculate enrichment broths, phosphate-buffered saline (PBS), apple juice and fruit slices. Injured *Salmonella* cells resuscitated and regained the ability to form colonies on selective agar (Xylose-Lysine-Tergitol® 4) if they were incubated in lactose broth (LB), universal pre-enrichment broth (UPB) or buffered peptone water (BPW), but not in tetrathionate broth, PBS or apple juice. The resuscitation occurred at a significantly ($P > 0{\cdot}05$) faster rate in UPB than in LB or BPW. The resuscitation also occurred on the surfaces of fresh-cut cucumber at 20°C, but not at 4°C.

Conclusions: Acid-injured *Salmonella* cells resuscitated in nonselective enrichment broths at different rates, but not in selective enrichment broth, apple juice, PBS or on fresh-cut apple.

Significance and Impact of the Study: Pre-enrichment of food samples in UPB prior to selective enrichment is recommended. Injured *Salmonella* cells have the ability to resuscitate on fresh-cut surfaces of cucumber when stored at abusive temperatures.

Keywords: acid injury, apple, cucumber, enrichment broth, fresh cut, juice, resuscitation, *Salmonella*.

INTRODUCTION

Use of organic acids such as acetic acid (AA) for decontamination of beef carcasses (Anderson *et al.* 1988) and fresh produce (Beuchat 1998) has been previously reported or suggested. The efficacy of AA as a disinfectant is primarily due to its bactericidal and injuring activities. Upon exposure to 2·0% AA for 5 min, over 65% of *Salmonella enterica* serovar Typhimurium remaining on beef carcasses were injured as indicated by their inability to form colonies on selective agar media such as Bismuth Sulfite Agar (Dickson 1992). Similarly, more than 30% of *S. enterica* serovar Mbandaka remaining on apple slices after treatment with 2·4% AA for 5 min were injured (Liao *et al.* 2003). The natural occurrence of injured or stressed *S. enterica* on contaminated foods is well recognized (Busta 1976). To improve the detection of *S. enterica* in foods, it has been recommended that samples be incubated in pre-enrichment broth before selective enrichment to allow those injured bacteria present to repair (Andrews 1989).

A great deal of effort has been made to develop optimal media and conditions for the recovery of *S. enterica* and other bacterial pathogens in foods (Ray 1979; Mackey 2000). Several pre-enrichment broths including lactose broth (LB), buffered peptone water (BPW) and universal pre-enrichment broth (UPB) for isolation of *S. enterica* are currently

Correspondence to: Ching-Hsing Liao, Eastern Regional Research Center, Agricultural Research Service, US Department of Agriculture, 600 E. Mermaid Lane, Wyndmoor, PA 19038, USA (e-mail: cliao@errc.ars.usda.gov).

**Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.*

available. Use of enrichment broths for improving the resuscitation of injured bacteria caused by heat, freezing, or irradiation has been investigated and documented (Andrews 1989). However, presently very little is known about the resuscitation of acid-injured bacteria under different incubation conditions in broth media, in fruit juices and on cut surfaces of fresh produce.

A better understanding of resuscitation of acid-injured *S. enterica* under different conditions could provide a scientific basis for developing a more effective method for isolation of pathogens and for assessing the safety risk associated with injured *S. enterica* on fresh produce or in fruit juices. The objectives of this study were to: (i) compare the efficacy of four selected enrichment broths and storage conditions for resuscitation of acid-injured *S. enterica*; and (ii) determine the ability of injured cells to resuscitate in apple juice and on the surfaces of fresh-cut cucumber and apple.

MATERIALS AND METHODS

Bacterial strains and culture media

Three produce-associated strains of *S. enterica* including serovars Mbandaka, Chester and Newport were used in this study. *Salmonella enterica* serovars Mbandaka (strain S7) and Newport (strain S10) isolated previously from alfalfa seeds and serovar Chester (strain S14) from cantaloupe were obtained from our culture collection. Selective and nonselective media including brain–heart infusion agar (BHIA), Xylose-Lysine-Tergitol® 4 (XLT4) agar, LB, BPW, UPB and tetrathionate (TT) broth were obtained from Difco/BD Systems (Sparks, MD, USA). Phosphate-buffered saline (PBS, 75 μmol, pH 7·1) containing KH_2PO_4 (15·44 μmol l^{-1}), NaCl (1·55 mmol l^{-1}) and Na_2HPO_4 (27·09 μmol l^{-1}) was obtained from the Invitrogen Corporation (Carlsbad, CA, USA). Bacteria grown on BHIA at 37°C for 18–20 h were used throughout this study.

Preparation and enumeration of acid-injured bacteria

The method used for preparation of acid-injured *S. enterica* have been described previously (Liao *et al.* 2003). Briefly, bacteria grown on BHIA at 37°C for 18 h were harvested using a sterile loop and suspended in PBS to make a suspension with an absorption unit of 1·00 ± 0·01at 600 nm (equivalent to *c.* $1{\cdot}0 \times 10^9$ CFU ml^{-1}). The suspension containing a single strain of serovar Mbandaka at the concentration of *c.* $1{\cdot}0 \times 10^9$ CFU ml^{-1} or a mixture of three serovars (Mbandaka, Chester and Newport) of approximately equal numbers ($0{\cdot}3$–$0{\cdot}4 \times 10^9$ CFU ml^{-1} per serovar) was exposed to 2·4% AA for 7 min. Treated samples were serially diluted and plated on BHIA and XLT4 to determine the injury rate. Injured bacteria were enumerated based on their ability to form colonies on nonselective BHIA but not on selective XLT4 medium. The difference in bacterial counts as determined on BHIA and on XLT4 was considered as the number of bacteria injured. The percentage of bacteria injured was calculated based on the formula:

$$\%\text{ of cells injured} = \frac{\text{CFU ml}^{-1}\text{of AA-treated sample as determined on BHIA} - \text{CFU ml}^{-1}\text{of AA-treated sample as determined on XLT4}}{\text{CFU ml}^{-1}\text{of AA-treated sample as determined on BHIA}} \times 100\%.$$

Resuscitation of acid-injured *Salmonella* in enrichment broth, apple juice and PBS

Injured cells of *S. enterica* serovar Mbandaka prepared as described above were used to inoculate UPB, BPW, LB, TT, PBS and apple juice to an initial density of *c.* 10^7 CFU ml^{-1} as determined on BHIA (injured + uninjured) or 10^3 CFU ml^{-1} as determined on XLT4 (uninjured only). The majority (99·99%) of the cells used for inoculation were injured, which was consistent with the estimate reported previously (Liao *et al.* 2003). Apple juice (Mott's brand) was purchased from local grocers and the average pH of the juice was determined to be 3·5 ± 0·1. Inoculated UPB, LB, TT, PBS and apple juice were incubated at 20°C without shaking for 4, 8 and 24 h. Resuscitation of the injured cells was indicated by a steady increase in the bacterial count as determined on XLT4 or by a steady decrease in the percentage of injured cells following incubation. To determine the effect of incubation temperature on resuscitation, BPW was inoculated with AA-injured *S. enterica* serovar Mbandaka and then incubated at 4, 20 and 35°C for 24 h. The samples were taken at 4, 8 and 24 h after incubation and the change in the percentage of injured cells in total population was determined.

Resuscitation of acid-injured *Salmonella* on the surfaces of fresh-cut cucumber and apple

The filter membrane method previously described for resuscitation of *Erwinia* on the surfaces of fresh-cut cucumber or apple (Liao and Shollenberger 2004) was used for study of the effect of fruit type on resuscitation of acid-injured *Salmonella*. Unwaxed apples (cv. Golden Delicious) and cucumbers were purchased from the local grocers. After surface-sanitization with 85% ethanol, fruits were cut open with a sterile knife. The pH of fresh-cut apple and cucumber were determined using a surface pH electrode (Sensorex Inc., Garden Grove, CA, USA). A sterile nitrocellulose membrane (0·45 μm, 25 mm in diameter; Millipore Inc., Bedford, MA, USA) was placed on the surfaces of fresh-cut cucumber or apple and inoculated with 50 μl of acid-injured *S. enterica* suspension containing a single serovar Mbandaka or a mixture of three serovars (Mbandaka, Chester and Newport). After incubation at 4 or 20°C for 4, 8 and 24 h, two membranes at each time point were removed and each membrane was immersed in 5 ml of PBS and vigorously shaken for 30 s. Total population and the percentage of injured cells in the suspension were determined by plating on BHIA and XLT4.

Statistical analysis

The changes in the populations of injured cells were analysed by performing analysis of variance to determine the effect of incubation times. Differences in bacterial populations between incubation times were subjected to the Bonferroni LSD mean separation procedure (Miller 1981) at the significance level $P = 0·05$.

RESULTS

Resuscitation of acid-injured *Salmonella* in nonselective enrichment broths

The relative effectiveness of three nonselective broths (LB, UPB and BPW) for resuscitation of AA-injured cells of *S. enterica* serovar Mbandaka was investigated. The changes in the percentage of injured *S. enterica* in the total population were determined after incubation at 20°C for 4, 8 and 24 h. The proportion of *S. enterica* serovar Mbandaka cells capable of forming colonies on XLT4 steadily increased with the time of incubation in all three nonselective broth media (Table 1). The percentage of injured cells capable of forming colonies only on BHIA but not on XLT4 steadily decreased from >90% to <30% after incubation for 8 h. In addition, resuscitation appeared to occur at a significantly faster rate ($P > 0·05$) in UPB than in LB or BPW (Table 1). After incubation at 20°C for 4 h, 60% of injured cells that were placed in UPB resuscitated when compared with 40–42% of injured cells that were placed in LB or BPW respectively. Similarly, after incubation at the same temperature for 8 h, 84% of injured cells that were incubated in UPB resuscitated when compared with 72% of injured cells that were incubated in LB or BPW.

Resuscitation of acid-injured *Salmonella* in apple juice, PBS, TT and BPW as affected by incubation temperature

Resuscitation of AA-injured cells of *S. enterica* serovar Mbandaka in apple juice (pH 3·5 ± 0·1), PBS, and TT was first examined after incubation of the inoculated samples at 20°C for 4, 8 and 24 h. No significant change in the percentage ($P > 0·05$) of the injured cells in total population

Table 1 Resuscitation of acid-injured cells of *Salmonella enterica* serovar Mbandaka in buffered peptone water (BPW), universal pre-enrichment broth (UPB) and lactose broth (LB) at 20°C*

	UPB (log CFU ml^{-1})			BPW (log CFU ml^{-1})			LB (log CFU ml^{-1})		
Incubation time (h)	BHIA	XLT4	% injury	BHIA	XLT4	% injury	BHIA	XLT4	% injury
0	6·8 ± 0·4†	4·4 ± 0·2	99Aa	7·2 ± 0·3	5·0 ± 0·2	99Aa	6·3 ± 0·1	4·3 ± 0·2	99Aa
4	7·3 ± 0·2	7·1 ± 0·3	40Bb	7·1 ± 0·2	6·7 ± 0·3	58Ba	6·7 ± 0·2	6·3 ± 0·1	60Ba
8	7·9 ± 0·3	7·8 ± 0·2	16Cb	7·9 ± 0·1	7·7 ± 0·3	28Ca	7·8 ± 0·5	7·3 ± 0·3	28Ca
24	9·1 ± 0·4	9·2 ± 0·3	<1Db	9·4 ± 0·3	9·3 ± 0·2	7Da	9·2 ± 0·3	9·1 ± 0·2	<1Db

*Injured bacteria were used to inoculate BPW, UPB, and LB to the initial densities shown (0-h incubation). % injury was determined based on the formula described (see Materials and methods). BHIA, brain heart infusion agar; XLT4, Xylose Lysine Tergitol® 4.

†The values represent the average data from three experiments with two replicates per experiment ± SD. Within a row or a column as indicated by upper or lower case letters, respectively, means (% injury) with no letter in common are significantly different ($P > 0·05$) by the Bonferroni LSD separation technique.

was observed after incubation in apple juice, PBS or TT broth for 24 h (Table 2). This indicated that apple juice, PBS and TT broth failed to support the resuscitation of injured bacteria. To investigate the effect of incubation temperature on resuscitation, BPW was inoculated with AA-injured *S. enterica* serovar Mbandaka and then incubated at 4, 20 or 35°C for up to 24 h. When the samples were incubated at 4°C, no changes in the total cell population and in the percentage of injured cells were observed after incubation for 24 h (Table 3). However, resuscitation of the injured cells were observed with samples that were incubated at 20 or 35°C. The percentage of injured cells in the total population declined rapidly from over 90% to *c.* 10% after placing the samples at 20 or 35°C for 24 h. In addition, the total population of *S. enterica* serovar Mbandaka also increased from *c.* 7 to 9 log CFU ml^{-1}. No significant difference ($P > 0.05$) in the recovery rate was observed with the samples that were incubated at 20 or 35°C (Table 3).

Resuscitation of acid-injured *Salmonella* on the surfaces of fresh-cut cucumber and apple

When filter membranes containing AA-injured *S. enterica* serovar Mbandaka cells were incubated on the surfaces of fresh-cut cucumber (pH 6·0 ± 0·1) at 20°C for 24 h, total viable cell counts (injured + uninjured) as determined on BHIA steadily increased from 4·8 to 7·3 log CFU per membrane. The number of the uninjured cell as indicated by their ability to form colonies on XLT4 increased from 3·5 to 7·3 log CFU per membrane and the percentage of the injured cells declined from 95% to 5% after 24 h of incubation (Table 4). Similar results were obtained when fresh-cut cucumber was inoculated with a mixture of three serovars (Mbandaka, Chester and Newport) (data not shown). However, when filter membranes containing AA-injured *S. enterica* serovar Mbandaka cells were placed on the surfaces of fresh-cut apple (pH 4·0 ± 0·1), the total cell counts on membranes as determined on BHIA decreased from 5·1 to 4·3 log CFU per membrane and the number of uninjured bacterial cells (based on their ability to form colonies on XLT4) decreased from 4·0 to 2·9 log CFU per membrane (Table 4). Furthermore, the percentage of injured bacterial cells on apple surfaces showed little change (from 92% to 96%) during the 24-h incubation period. No resuscitation was observed with the membranes that were placed on fresh-cut cucumber and incubated at 4°C (data not shown).

Table 2 Resuscitation of acid-injured cells of *Salmonella enterica* serovar Mbandaka in apple juice, phosphate-buffered saline (PBS) and tetrathionate selective enrichment broth (TT) at 20°C*

	Juice			PBS			TT		
Incubation time (h)	BHIA	XLT4 (log CFU ml^{-1})	% injury	BHIA	XLT4 (log CFU ml^{-1})	% injury	BHIA	XLT4 (log CFU ml^{-1}l)	% injury
0	7·1 ± 0·1†	5·8 ± 0·3	95a	7·3 ± 0·1	5·9 ± 0·2	96a	6·5 ± 0·2	4·1 ± 0·1	99a
4	6·8 ± 0·3	5·8 ± 0·2	91a	7·3 ± 0·2	5·0 ± 0·3	94a	6·2 ± 0·1	4·8 ± 0·3	96a
8	6·8 ± 0·4	5·8 ± 0·1	90a	7·2 ± 0·3	5·0 ± 0·1	93a	6·1 ± 03	4·3 ± 0·5	98a
24	6·0 ± 0·3	4·8 ± 0·2	93a	6·9 ± 0·1	5·6 ± 0·4	95a	6·3 ± 0·2	4·2 ± 0·2	99a

*See Table 1 footnotes for abbreviation of BHIA and XLT4 and for calculation of % injury.
†The values represent the average data from three experiments with two replicates per experiment ± SD. Within a column, means with no letter in common are significantly different ($P > 0.05$) by the Bonferroni LSD separation technique.

Table 3 Resuscitation of acid-injured cells of *Salmonella enterica* serovar Mbandaka in buffered peptone water (BPW) as affected by incubation temperature*

	4°C			20°C			35°C		
Incubation time (h)	BHIA	XLT4 (log CFU ml^{-1})	% injury	BHIA	XLT4 (log CFU ml^{-1})	% injury	BHIA	XLT4 (log CFU ml^{-1})	% injury
0	6·9 ± 0·2†	5·8 ± 0·1	93a	6·9 ± 0·2	5·6 ± 0·1	95a	6·9 ± 0·3	5·5 ± 0·1	96a
4	6·9 ± 0·4	5·7 ± 0·3	93a	6·9 ± 0·3	6·5 ± 0·2	60b	6·9 ± 0·2	6·4 ± 0·3	72b
8	6·9 ± 0·2	5·7 ± 0·2	94a	7·0 ± 0·4	6·9 ± 0·3	30c	7·6 ± 0·4	7·4 ± 0·2	29c
24	6·9 ± 0·1	5·8 ± 0·3	91a	9·0 ± 0·3	9·0 ± 0·2	<1d	9·5 ± 0·1	9·5 ± 0·3	<1d

*See Table 1 footnotes for abbreviation of BHIA and XLT4 and for calculation of % injury.
†See Table 2 footnote †.

Table 4 Resuscitation of acid-injured cells of *Salmonella enterica* serovar Mbandaka on the surfaces of fresh-cut cucumber and apple at 20°C*

	Cucumber			Apple		
Incubation time (h)	BHIA	XLT4 (log CFU per membrane)	% injury	BHIA	XLT4 (log CFU per membrane)	% injury
0	4·8 ± 0·2†	3·5 ± 0·3	95a	5·1 ± 0·3	4·0 ± 0·1	93a
4	5·2 ± 0·1	4·9 ± 0·4	50b	4·9 ± 0·2	3·7 ± 0·3	94a
8	6·1 ± 0·3	6·0 ± 0·1	30c	4·1 ± 0·4	3·3 ± 0·4	92a
24	7·3 ± 0·2	7·3 ± 0·2	5d	4·3 ± 0·1	2·9 ± 0·3	96a

*See Table 1 footnotes for abbreviation of BHIA and XLT4 and for calculation of % injury.
†See Tables 1–3 footnote †.

DISCUSSION

It has been previously demonstrated that application of UPB increases the detection of *S. enterica* in apple and orange juices (Hammack *et al.* 2001, 2002) and increases the detection of *Escherichia coli* O157:H7 in ground beef (Zhao and Doyle 2001). Pre-enrichment in UPB is also useful for simultaneous detection of heat-injured *Salmonella* and *Listeria* in chicken and hot dogs (Bailey and Cox 1992). Here, we report that acid-injured *S. enterica* cells resuscitated at a much faster rate in UPB than in LB or BPW. Thus, UPB thus represents a preferred broth for enrichment of injured or stressed *Salmonella* from fresh produce and juice products as well. We have previously demonstrated that two repeated enrichment steps in BPW can increase the isolation of *Salmonella* from alfalfa seeds implicated in previous disease outbreaks (Liao and Fett 2003). An adequate enrichment of the sample in UPB or BPW is therefore critical for isolation of the extremely low number of *Salmonella* from fresh produce or sprouting seeds (Inami *et al.* 2001). However, direct enrichment of the samples, especially those possibly containing the stressed or injured *Salmonella*, in selective broth such as TT should be avoided. In the present study, inoculation of AA-injured cells in TT not only inhibited their resuscitation but also accelerated their death as indicated by a gradual decline in the total population of *Salmonella* following incubation. Wells and Butterfield (1997) also showed that direct enrichment of the samples in TT reduced the isolation of *Salmonella* from soft-rotted produce. However, Beuchat and Scouten (2004) found that direct enrichment of the samples in TT did not seem to affect the recovery of *Salmonella* from inoculated cantaloupe.

The ability of injured cells of *S. enterica* serovar Bareily (Blankenship 1981) and *E. coli* (Przybylski and Witter 1979) to resuscitate in potassium or sodium phosphate buffered solutions has been previously reported. However, we were unable to confirm the resuscitation of AA-injured *S. enterica* serovar Mbandaka in PBS (Table 2). As noted earlier by Ray (1979), the recovery from bacterial injury may not require the *de novo* synthesis of protein, DNA or RNA, but may involve the synthesis of ATP. By placing injured bacteria in PBS rather than in rich broth, the cells may not be able to generate the energy force (ATP) required for the repair process. It is also possible that the ability or inability of injured bacteria to resuscitate in buffered solutions may be dependent on the extent of injury and incubation conditions (Mackey 2000). In this study, we demonstrated that the repairing of injury is critically affected by the incubation temperature. Acid-injured cells of *S. enterica* serovar Mbandaka were able to resuscitate at 20 or 35°C, but not at 4°C. Storage of fresh and fresh-cut produce at refrigeration temperature represents a simple and convenient practice for preventing the proliferation of injured and uninjured pathogens.

Incubation of injured *Salmonella* in apple juice or on fresh-cut apple inhibited resuscitation and accelerated the death of injured bacteria as indicated by a marked decline in the total population of *Salmonella*. This finding is in agreement with a previous report by Parish *et al.* (1997), who showed that *Salmonella* cells survived in apple or orange juice for only one to several weeks. While no resuscitation of acid-injured *Salmonella* on the acidic surfaces of fresh-cut apple (pH 4·0 ± 0·1) was detected, resuscitation of acid-injured *Salmonella* on the surfaces of fresh-cut cucumber which have a neutral pH (6·0 ± 0·1) was observed. This is the first demonstration of resuscitation of injured bacterial pathogens on fresh-cut produce. These results suggest that the safety risk associated with injured pathogens on fresh produce cannot be ignored and a pre-enrichment treatment in UPB may improve the isolation of injured *Salmonella* from contaminated fresh produce.

ACKNOWLEDGEMENTS

We thank Lisa Shollenberger for technical assistance, John Phillips for assistance in statistical analysis and Jim Smith for critical review, and suggestions during the preparation of this manuscript.

REFERENCES

Anderson, M.E., Huff, H.E., Naumann, H.D. and Marshall, R.T. (1988) Counts of six types of bacteria on lamb carcasses dipped or sprayed with acetic acid at 25°C or 55°C and stored vacuum packaged at 0°C. *J Food Prot* **51**, 874–877.

Andrews, W.H. (1989) Methods for recovering injured "classical" enteric pathogenic bacteria (*Salmonella*, *Shigella*, and enteropathogenic *Escherichia coli*) from foods. In *Injured Index and Pathogenic Bacteria: Occurrence and Detection in Foods, Water and Feeds* ed. Ray, B. pp. 55–113. Boca Raton, FL, USA: CRC Press.

Bailey, J.S. and Cox, N.A. (1992) Universal pre-enrichment broth for the simultaneous detection of *Salmonella* and *Listeria* in foods. *J Food Prot* **55**, 256–259.

Beuchat, L.R. (1998) *Surface Decontamination of Fruits and Vegetables Eaten Raw: A Review*. Geneva: Food Safety Unit, World Health Organization, WHO/FSF/FOS/982.

Beuchat, L.R. and Scouten, A.J. (2004) Factors affecting survival, growth, retrieval of *Salmonella* Poona on intact and wounded cantaloupe rind and in stem scar tissue. *Food Microbiol* **21**, 683–694.

Blankenship, L.C. (1981) Some characteristics of acid injury and recovery of *Salmonella bareilly* in a model system. *J Food Prot* **44**, 73–77.

Busta, F.F. (1976) Practical implications of injured microorganisms in food. *J Milk Food Technol* **39**, 138–145.

Dickson, J.S. (1992) Acetic acid action on beef tissue surfaces contaminated with *Salmonella typhimurium*. *J Food Sci* **57**, 297–301.

Hammack, T.S., Amaguaña, R.M. and Andrews, W.H. (2001) An improved method for the recovery of *Salmonella* serovars from orange juice using universal pre-enrichment broth. *J Food Prot* **64**, 659–663.

Hammack, T.S., Johnson, M.L., Jacobson, A.P. and Andrews, W.H. (2002) Enhanced recovery of *Salmonella* from apple cider and apple juice with universal pre-enrichment broth. *J AOAC Int* **85**, 384–387.

Inami, G.B., Lee, S.M.C., Hogue, R.W. and Brenden, R.A. (2001) Two processing methods for the isolation of *Salmonella* from naturally contaminated alfalfa seeds. *J Food Prot* **64**, 1240–1243.

Liao, C.-H. and Fett, W.F. (2003) Isolation of *Salmonella* from alfalfa seed and demonstration of impaired growth of heat-injured cells in seed homogenates. *Int J Food Microbiol* **82**, 245–253.

Liao, C.-H. and Shollenberger, L.M. (2004) Enumeration, resuscitation, and infectivity of the sublethally injured *Erwinia* cells induced by mild acid treatment. *Phytopathology* **94**, 76–81.

Liao, C.-H., Shollenberger, L.M. and Phillips, J.G. (2003) Lethal and sublethal action of acetic acid on *Salmonella* cells *in vitro* and on cut surfaces of apple slices. *J Food Sci* **68**, 2793–2798.

Mackey, B.M. (2000) Injured bacteria. In *The Microbiological Safety and Quality of Food*, Vol. II ed. Lund, B.M., Baird-Parker, T.C. and Gould, G.W. pp. 315–341. Gaithersburg, MD, USA: Aspen Publishers.

Miller, R.G. (1981) *Simultaneous Statistical Inference*, 2nd edn, p. 299. New York, NY: Springer-Verlag.

Parish, M.E., Narciso, J.A. and Friedrich, L.M. (1997) Survival of salmonellae in orange juice. *J Food Safety* **17**, 273–281.

Przybylski, K.S. and Witter, L.D. (1979) Injury and recovery of *Escherichia coli* after sublethal acidification. *Appl Environ Microbiol* **37**, 261–265.

Ray, B. (1979) Methods to detect stressed microorganisms. *J Food Prot* **42**, 346–355.

Wells, J.M. and Butterfield, J.E. (1997) *Salmonella* contamination associated with bacterial soft rot of fresh fruits and vegetables in the marketplaces. *Plant Dis* **81**, 867–872.

Zhao, T. and Doyle, M.P. (2001) Evaluation of universal pre-enrichment broth for growth of heat-injured bacteria. *J Food Prot* **64**, 1751–1755.